POLYSACCHARIDE COMPOSITIONS, PREPARATION AND USES

BACKGROUND OF THE INVENTION

This invention relates to polysaccharide compositions of the gel-forming beta-1,3-glucan type and to methods of preparing and using solutions and gels of the polysaccharides. The novel gels of the invention provide added benefits in established fields and open up a myriad of new applications for the polysaccharides in foods, industrial products and processes, and in a broad spectrum of cosmetics, pharmaceutical and biomedical applications.

The beta-1,3-glucan polysaccharides are unique because they are the only known polysaccharides, other than agarose, which not only are neutral but also form nearly transparent, relatively firm hydrogels at low concentrations. These polysaccharides are exo-3-glucan polymers composed almost exclusively of beta-(1, 3)-glucosidic linkages. The beta-1,3-glucan polysaccharides widely distributed in nature as components of yeasts (cell walls), land and sea plants, and seeds (also as cell walls), and can be biologically produced, for example by microbial fermentation. Microorganisms which produce the beta-1,3-glucans include bacteria of the genera Alcaligenes, Agrobacterium and Streptococcus of which the species *Alcaligenes faecalis, Agrobacterium radiobacter, Agrobacterium rhizogenes* and *Streptococcus mutans,* including variants and mutations thereof, are the most widely known. The bacterially produced beta-1,3-glucan polysaccharides are also known as curdlans, Takeda polysaccharide 13140, scleroglucans, succinoglucans, schizophyllans, pachymans (from the fungus *Poria cocos*), paramylons and by other designations.

The beta-1,3-glucan polysaccharides are extensively described in the patent and technical literature, such as U.S. Pat. Nos. 3,754,925 and 3,822,250; "Production, Properties, And Application of Curdlan", T. Harada, in *Extracellular Microbial Polysaccharides,* ACS Symposium Series No. 45, American Chemical Society, Washington, D.C., 1977, 265–283 (1977); and "Curdlan: Its Properties And Production In Batch And Continuous Fermentations", K. R. Philipps and H. G. Lawford, *Progress in Industrial Microbiology,* ed. M. E. Bushell, 18, 201–229 (1983), Elsevier Scientific Publishing (Amsterdam). The last article and U.K. patent application No. 2090847A, published July 21, 1982, describe a two-stage continuous process for producing curdlan-type polysaccharides from microorganisms such as *Alcaligenes faecalis* var. *myxogenes* (ATCC 31749 and ATCC 31750). The curdlan-type polysaccharides are preferred for use in the present invention.

Despite the many studies devoted to production and uses of the beta-1,3-glucan polysaccharides, these materials have not been commercialized. A primary reason for this is the lack of a convenient and economical method for forming gels. Known gel-forming processes include (1) heating a swollen, aqueous paste of the polysaccharide powder, resulting in a semi-continuous, particulate gel; (2) heating an aqueous suspension of the polysaccharide; (3) acidifying an alkaline solution of the polysaccharide by dripping the solution into aqueous hydrogen chloride (U.S. Pat. No. 3,899,480), by treating with a slow release acid encapsulated in a polymeric matrix (British Pat. No. 1,500,456) or by treating with acid anhydride vapor such as gaseous carbon dioxide (U.S. Pat. No. 4,012,333); (4) diluting with water a solution of the polysaccharide in an organic solvent such as dimethyl sulfoxide; and (5) dialyzing alkaline solutions against various solutions.

All of these methods are unduly cumbersome and/or uneconomical on a commercial scale. Moreover, they do not provide the good control over polysaccharide concentration, gel structure and ionic environment throughout the gel, which are required in many applications of the gels, particularly in the treatment of sensitive biological materials.

SUMMARY OF THE INVENTION

One aspect of the invention is a novel beta-1,3-glucan polysaccharide gel, characterized by (a) coherent, uniform, non-particulate structure as determined visually and by continuous, uniform staining with Aniline Blue, and (b) substantially uniform pH throughout the gel structure immediately upon formation of the gel.

By "coherent" in this specification is meant a continuous, cohesive appearance, free of undissolved particles (although microscopic fibrils may be present) and swollen masses, both to the unaided eye and when stained with Aniline Blue. In contrast, prior beta-1,3-glucan polysaccharide gels, such as those prepared from suspensions, as in U.S. Pat. Nos. 3,754,925 and 3,822,250, are characterized by phase discontinuity and pockets of swollen, undissolved particles, as made more evident by mottling high-lighted by Aniline Blue staining. The coherency reflects more homogeneous polysaccharide concentration and structure, thereby enhancing the usefulness of the gels as media for supporting, separating, transforming or treating biological materials.

The substantially uniform pH throughout the gels of the invention immediately upon formation contrasts wih the character of the gels prepared by the method of U.S. Pat. No. 4,012,333 to Towle. In Towle, an alkaline solution of a beta-1,3-glucan-type polysaccharide is gelled by exposure to an atmosphere of a gaseous acid anhydride such as carbon dioxide. The gelling technique requires slow diffusion of the gaseous acid anhydride through the polysaccharide solution, with the result that a pH gradient (for example, from about 4 to 8) occurs in the gel. An adverse consequence of the need to hold a solution at an alkaline pH is that the gels cannot be used to support materials such as cells, enzymes, antibodies, and the like, which are sensitive to alkaline pH or to a pH range.

In another aspect of the invention, a convenient low cost method (hereinafter sometimes called the "Critical Temperature Neutralization" or "CTN" method) has been found for preparing solutions and gels of the beta-1,3-glucan polysaccharides based upon the discovery of a narrow temperature range where neutralization or reduction of the pH of an alkaline solution of the polysaccharides to 10.5 or lower does not immediately induce gellation. However, the solution, once formed in the critical temperature range and having a pH of 10.5 or lower, can be conveniently gelled either by cooling to form a thermally reversible ("cold set" or "low set") gel, or by further heating to form a thermally irreversible ("heat set" or "high set") gel.

In the CTN method for preparing solutions and gels of the invention, a suitable beta-1,3-glucan polysaccharide is dissolved in an aqueous alkaline medium (pH over 10.5) at a temperature at or below about 55° C. The resulting solution is then heated, if necessary, to the